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ORIGINAL ARTICLE

Microcapillary Electrophoresis Chip Device Integrated with Micro Focusing Lens Structures and Its Biomedical Applications

Suz-Kai Hsiung[1]*, Gwo-Bin Lee[2], Che-Hsin Lin[3], Chun-Hong Lee[1]

[1]*Automation Fluid System Developmental Center, Fooyin University, Kaohsiung, Taiwan*
[2]*Department of Engineering and Science, National Cheng Kung University, Tainan, Taiwan*
[3]*Department of Mechanical and Electromechanical Engineering, National Sun Yat-Sen University, Kaohsiung, Taiwan*

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In this paper, we present a micro-electro-mechanical-system based on a microcapillary electrophoresis chip device integrated with optical detection components, including a micro-focusing lens structure and buried optic fibers. This is a promising approach to enhance the optical signal of the laser-induced fluorescence system for biomedical detection applications. This study utilized microcapillary electrophoresis (micro-CE) chips with two specific polymer materials, polymethylmethacrylate (PMMA) and polydimethylsiloxane (PDMS). Both are capable of performing multiple-wavelength fluorescence detection by using integrated optic components. These include multimode optic fiber pairs and a micro-focusing-lens structure, embedded downstream of the separation channel. For detection purposes, the fluorescence signals are enhanced by positioning micro-focusing-lens structures at the outlets of the excitation fibers and the inlets of the detection fibers. In this study, two types of micro-focusing-lens are proposed—fixed-focal-length and controllable micro-lenses. They are made from different materials—PMMA and PDMS, respectively. With regard to the fixed-focal-length micro-lenses, the profile of the micro-lens curve can be formed by the defined master mold with specific temperatures and pressures. With regard to the controllable micro-lens design, deformations of the two flexible surfaces can be generated after pressurized index-matching fluid is injected into the pneumatic side-chambers. The side-chambers can be deflected as a double convex lens to focus both the excitation light source and the fluorescent emission signal. Experimental results revealed that the power amplitude of the excitation laser light can be enhanced by up to 5.4 fold. Fluorescein isothiocyanate, dye labeled protein samples and DNA markers are then utilized for micro-CE chip testing. The results indicated that signal amplitude can be enhanced from 1.7 to 2.6 fold when compared with cases without the micro-lens. According to the experimental results, the developed device has a great potential to be integrated with other microfluidic devices for further biomedical applications.

Key Words: capillary electrophoresis; laser-induced fluorescence; MEMS; micro-lens; multi-wavelength

Introduction

Micro-electro-mechanical-system (MEMS)

MEMS technology has been an enabling technology in a variety of fields, including biotechnology, communications, optics, and many others. MEMS devices generally contain two principal components: a sensing or actuating element and a signal transduction unit.[1] MEMS technology has been increasingly employed in a number of industrial fields, including electromechanical engineering, biotechnology, and optoelectronics.[2,3] Bio-MEMS technology, which integrates the principles of molecular biology, genetic

*Corresponding author. Automation Fluid System Developmental Center, Fooyin University, Kaohsiung, Taiwan.
E-mail: ft040@mail.fy.edu.tw

information, and analytical chemistry with MEMS fabrication technologies, has been employed to develop a diverse range of microfluidic devices designed for the automatic reaction and analysis of biomedical samples. Miniaturized bio-devices are capable of performing many critical sample preparation and treatment functions on a single substrate in an automated fashion, including mixing, reaction, transportation, collection, separation, and detection.[4–8] Bio-MEMS technology provides the potential to develop miniaturized instrumentation which combines high orders of automation with short analysis times. The miniaturized bio-devices fabricated using this technology is generally referred to as "lab-on-a-chip" (LOC) devices. LOC systems typically have the advantages of shorter analysis times, reduced sample and reagent consumption, improved resolution, higher sensitivity, and low cost. LOC systems are eminently suitable for genetic engineering, environmental monitoring, and clinical medical diagnosis applications.[9,10]

Microcapillary electrophoresis chip device (micro-CE)

Micro-CE chips, fabricated using micromachining techniques, have considerable potential for use in the analysis of biological molecules such as proteins and DNA.[11] Compared with their conventional counterparts, micro-CE chips have a higher separation efficiency, physically smaller dimensions, reduced sample and reagent consumption, lower applied voltage requirements, and higher detection limits. Micro-CE chips generally employ a laser-induced fluorescence (LIF) detection mechanism since this method enhances both the selectivity and the sensitivity of the device.[12–16] Traditionally, chip-based capillary electrophoresis is performed under a high magnification microscope equipped with a single fluorescent light source and an optical detector. It has been reported that this scheme enables an excellent detection limit to be achieved (~10^{-11} M).[16] However, the LIF system is bulky and the experimental procedure tends to be inefficient since only one type of labeled sample can be detected within a single test run. Furthermore, achieving a precise alignment of the various optical components is time-consuming and complicated. Hence, a requirement exists to utilize micromachining technologies to fabricate a miniaturized optical detection system capable of executing the high-throughput analysis of biomolecules such as protein and DNA.

Integrated optical detection

Integrating the optical components within microfluidic devices offers a promising approach for reducing the size of the optical detection system. Several methods have been reported in the literature for the MEMS-fabrication of micro-optical-waveguides. An integrated optical circuit using multiple planar waveguides has also been demonstrated utilizing organomineral materials.[17] Buried optical waveguides circuits can be realized on glass substrates using a simple UV exposure process. Meanwhile, a straightforward hot-embossing technique has been presented for the fabrication of integrated planar waveguide structures on printed circuit boards.[18] However, these techniques generally involve the use of MEMS-non-compatible materials and processes and integrating these waveguides with glass-based microfluidic systems is challenging. Consequently, several studies have investigated the use of micro devices with integrated optical waveguides/fibers for the optical detection of biomolecules such as proteins and DNA. For example, Grewe et al[19] and Grosse et al[20] fabricated an optical leaky waveguide device in fused silica using wet chemical etching and bonding techniques. However, the leaky operation mode of this device resulted in optical losses in the waveguide. Liang et al presented a micro-CE chip with integrated optic fibers for absorbance detection applications. The proposed method was straightforward and provided a good optical connection between the microfluidic chip and the optical equipment. However, both approaches involved time-consuming manual processes such that the mass-production of these devices could be expensive and practical applications might be hindered. Hence, a microfluidic chip device integrated with buried optical fibers for absorbance detection was reported for bioanalytical applications.[21]

In addition, the issue of optical energy loss when using these optical waveguides and buried optical fibers still remains. This is primarily due to numerous connecting parts, such as the poor coupling efficiency between the excitation light source and the optical fibers. The intensity of the fluorescent signals is directly related to the power amplitude of the excitation light source so that optical energy loss leads to significant decrease of the on-chip detection method in terms of sensitivity when compared with the large-scale instrument. In order to address these problems, micro-lens structures have been reported, fabricated by various materials and technologies.[22,23] Recently, polymer micro-lenses have been widely utilized for light focusing of the excitation light source and fluorescent emission due to its good optical properties and easier fabrication process. The micro-lens can be placed on the top of the microfluidic device,[24–26] or inside micro channels.[27] Micro-lenses can be divided into two categories: fixed-focal-length and variable-focal-length micro-lenses.[28,29]

In this study, we have investigated two types of two-dimensional micro-focusing-lens structures—fixed-focal-length and controllable micro-lenses—for biomedical applications utilizing LIF detection. Pairs of multimode optical fibers are inserted and embedded into the microchip device through optical fiber channels for both the excitation light source and fluorescent emission transmitting. A pair of fixed-focal-length micro-lens structures is employed to increase the intensity of the excitation light source, thereby enhancing the fluorescence emission signals for detection purposes. Furthermore, in order to increase the focusing effect of the micro-lens structure, a pair of pneumatic side-chambers is placed between the optical fiber channel and the micro-CE channel as the controllable micro-lens structure. The intervals can be used to generate the controllable moving wall structure, so that the controllable micro-lens structure can be formed. The micro-lens can be deformed as a double convex lens to focus the light while the pressurized index-matching fluid is injected into the pneumatic side-chambers. The focal length can be then actively adjusted with different applied pressures of the index-matching fluid—the optical enhancing effect can be realized accordingly.

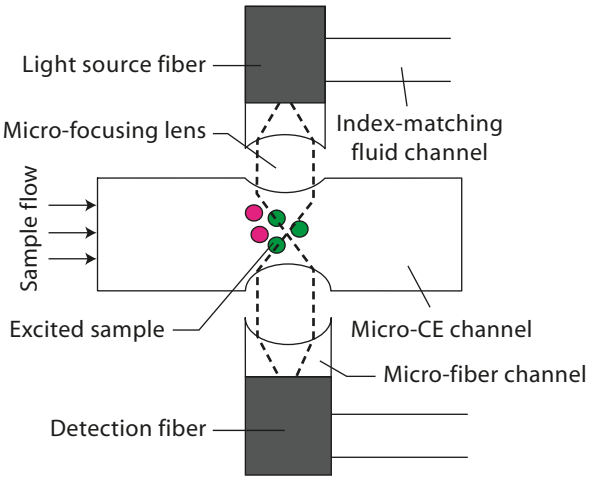

Figure 1 Schematic illustration (operating principle) of the high-throughput microcapillary electrophoresis chip device integrated with fixed focal length micro-lenses. As dye-labeled samples pass through the detection region they emit florescence signals because of excitation by appropriate laser sources guided through the embedded optical fibers. The fixed focal length micro-lens structures placed at the ends of the fibers provide a light focusing effect and significantly enhance the performance of the multi-wavelength laser-induced fluorescence detection device.

Materials and Methods

The schematic illustration of the operating principles of the micro-CE device integrated with fixed-focal-length micro-lens is shown in Figure 1. A pair of parallel multimode optic fibers is buried onto the micro-CE chip to support real-time fluorescent detection. Embedding the optic fibers in this way avoids the need for the conventional delicate optical alignment equipment and labor-intensive light coupling procedures. Samples labeled with specific fluorescent dyes are excited using specific light sources of appropriate wavelength from the two "light source" optic fibers. The excited fluorescence signals are then collected by the corresponding "detection" optic fibers embedded in the opposite channel wall. This arrangement allows the individual excitation and emission of multiple samples labeled with different kinds of fluorescent dyes without reciprocal influences. The proposed micro-focusing lenses formed using a hot-embossing process are located at the outlets of the excitation optic fibers and at the inlets of the detection fibers. These lenses can be utilized to focus both the excitation light source and fluorescent emission, and the amplitude of the fluorescence signal can be effectively enhanced. The light-transmission efficiency between the optic fibers has a significant impact on the detection results. Therefore, index matching

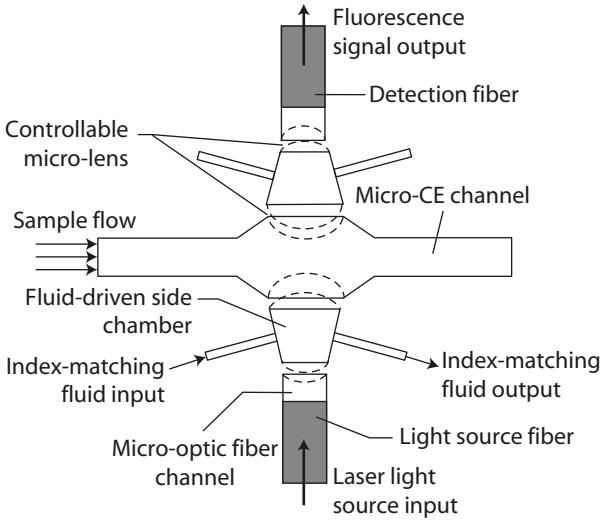

Figure 2 (A) Magnified view of integrated fixed focal length micro-lens and microcapillary electrophoresis channel. (B) Fluorescent dye in the sample flow channel excited by a laser source emitted from buried optic fiber.

between the end of the optic fiber and the sample flow channel is an important consideration. To address this problem, the current study designed side-channels to serve as inlets for a low-cost index matching fluid (ethanol).

Another component of the micro-focusing lens—the controllable micro-lens structure—is shown in Figure 2. The concept of a "controllable moving

wall" is adapted for the controllable micro-lens structure, which is capable of variable on-chip fluorescence detection. Briefly, it is constructed by the interval between a micro-channel and a pneumatic side-chamber. A deformation of the controllable moving wall structure can occur while the pressurized liquid is injected into the pneumatic side-chamber. Several microfluidic components are integrated onto the microchip device including the micro-CE channel, optic fiber channels, and a pair of pneumatic side-chambers. The intervals between the pneumatic side-chamber, micro-CE channel, and optical fiber channel are used to form the two surfaces of the controllable micro-lens structure—the outer and inner surfaces. The controllable micro-lens structure can be deflected while an index-matching fluid (ethanol), used to make uniform the refractive index of the controllable micro-lens structure (polydimethylsiloxane, PDMS; refractive index=1.44), is injected into the pneumatic side-chamber with a specific liquid pressure. The deformation of the controllable micro-lens can be precisely controlled by adjusting the pressure of the index-matching fluid. After the index-matching fluid is injected into the pneumatic side-chamber, the controllable micro-lens structure can be deflected as a double convex lens based on the designed geometry of the pneumatic side-chamber. The focal length of the micro-lens can then be actively adjusted by controlling the index-matching fluid pressures, so that varying focusing effects can be observed. In order to transmit the excitation light source and the emitted fluorescence signal, two optical fibers—the excitation source and detection fiber—are inserted into the chip device through the optical fiber channel. Due to the formation of the controllable micro-lens structure, the laser light source transmitted through the excitation source fiber can be enhanced and the emitted fluorescence signal amplitude of dye-labeled samples can be improved as well. The depth of the micro-lens structure is 100 μm, and the width of both outer and inner surfaces is 40 μm. The distance between two surfaces is 300 μm. The lengths of the outer and inner surfaces are 400 μm and 120 μm, respectively. The dimensions of the optical fiber channel are 120 μm in width and 100 μm in depth, respectively. Two multimode optical fibers with an outer diameter of 125 μm are inserted into the microfluidic chip and used to guide the excitation light source and collect fluorescent emission of dye-labeled sample in/out of the chip device. The dimensions of the micro-CE channel in the optical detection area are 200 μm in width and 100 μm in depth, respectively.

Figure 3A shows a photograph of the assembled micro-CE chip device integrated with the controllable micro-lens structure. Standard SU-8 master mold fabrication and replication processes were utilized to generate the micro structures including micro channels and pneumatic side-chambers on to the PDMS chip.[30] For the formation of the proposed microfluidic chip device, another unpatterned PDMS layer with a thickness of 5 mm was fabricated and bonded with the patterned PMDS layer to form a sealed microfluidic chip. After the

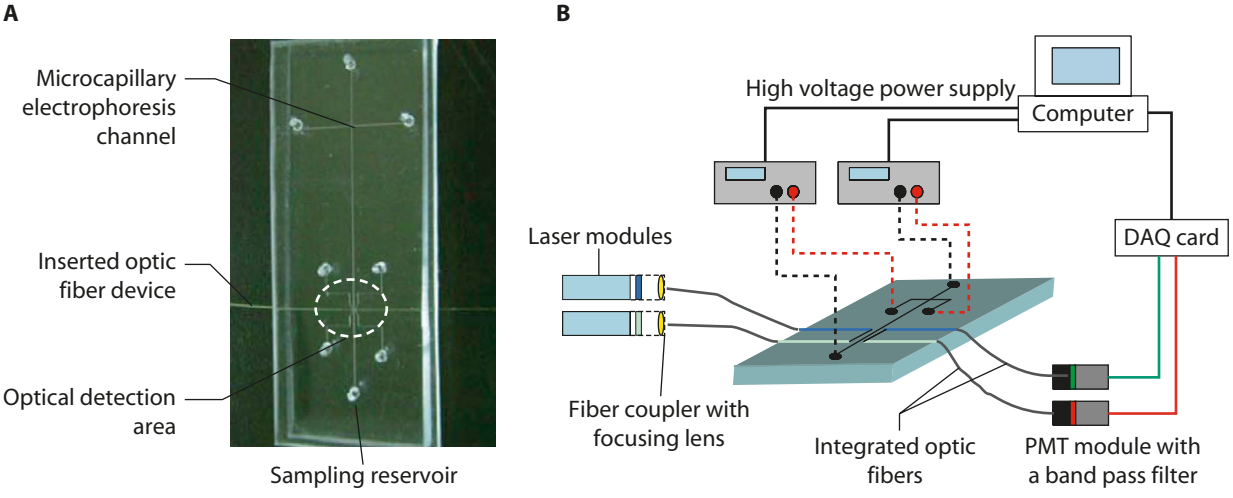

Figure 3 Schematic representation of the microcapillary electrophoresis device integrated with a controllable micro-lens structure. Several microfluidic components are integrated onto the microchip device, including a microcapillary electrophoresis channel, optical fiber channels, and a pair of pneumatic side-chambers. A deformation of the controllable moving wall structure can occur while the pressurized liquid is injected into the pneumatic side-chamber.

replicating the process for two PDMS layers, the chip device was assembled by bonding the two PDMS layers together using oxygen plasma treatment. The width and length of the chip is 3 cm and 8 cm, respectively; the length of the injection and separation channel is 2 cm and 6 cm, respectively. A pair of multimode optical fibers is then inserted into the micro-CE chip device through the optical fiber channels for the optical detection of the dye-labeled sample. The experimental setup of the micro-CE chip for the detection of fluorescence signals is shown in Figure 3B. Light sources used for the multi-wavelength fluorescence dye labeled sample excitation included an argon laser (488 nm, 50 mW; Laserphysics, Salt Lake City, UT, USA), a solid-state laser diode (543 nm, 10 mW; Onset Electro-Optics Co. Ltd., Taipei, Taiwan), and a He-Ne laser (632.8 nm, 5 mW; JDS Uniphase Corp., Milpitas, CA, USA). The laser sources were coupled into the inlet optic fibers using two individual optic fiber couplers (EF01A; Onset Electro-Optics Co. Ltd.) and a 10X objective focus lens. The emitted fluorescence signals were detected by a photomultiplier tube (PMT) module (C3830, R928; Hamamatsu Photonics K.K., Hamamatsu City, Shizuoka Prefecture, Japan). A band-pass filter was used in the front of the PMT to filter out the excitation laser light source. The amplified optical signals were then converted into analog signals and acquired by a commercially-available dual-channel 24-bit ADC module (Model 0224-2; SISC, Taipei, Taiwan) using a personal computer.

Results

Figure 4A shows a close-up view of the focusing lens within the integrated chip. To provide a focused light source, thereby increasing the amplitude of the signal and enhancing the detection performance, a focusing lens was positioned between the front-end of the optic fiber and the sample flow channel. Notably, the fiber channels are filled with an ethanol index-matching fluid, which significantly increases the sensing efficiency. Specifically, when this index-matching fluid is employed, the measured optical intensity is 2.71-fold greater than when the fluid is omitted. This fabrication process utilizes a simple and reliable hot-embossing technique, conducted at 130°C for 5 minutes to transfer the inverse image of the prefabricated master-mold onto the polymethylmethacrylate substrates. This generates the micro-channels and micro-focusing lens structures.

In the current study, the sample flow channel was initially filled with 10^{-5} M fluorescein isothiocyanate (FITC) fluorescent dye. The running buffer was a sodium borate solution of pH 9.2. A light source was then passed through the sample flow channel from the excitation optic fiber on one side of the channel to the detection optic fiber on the other. In this test, the light source was provided by a 50 mW Argon laser with a wavelength of 488 nm. Figure 4B shows the image of the excited fluorescent dye with focusing micro-lens effect. The micro-focusing-lens structure enhances the emitted fluorescence signal coupled into the optic fiber of the detection system by an order of 1.67 times.

A close-up view of the optical detection area composed of the micro-CE channel, two pneumatic side-chambers, and a pair of optical fiber channels is schematically shown in Figure 5A. In order to provide a focused light source to enhance signal amplitude for fluorescence detection, the pneumatic side-chamber was placed between the front

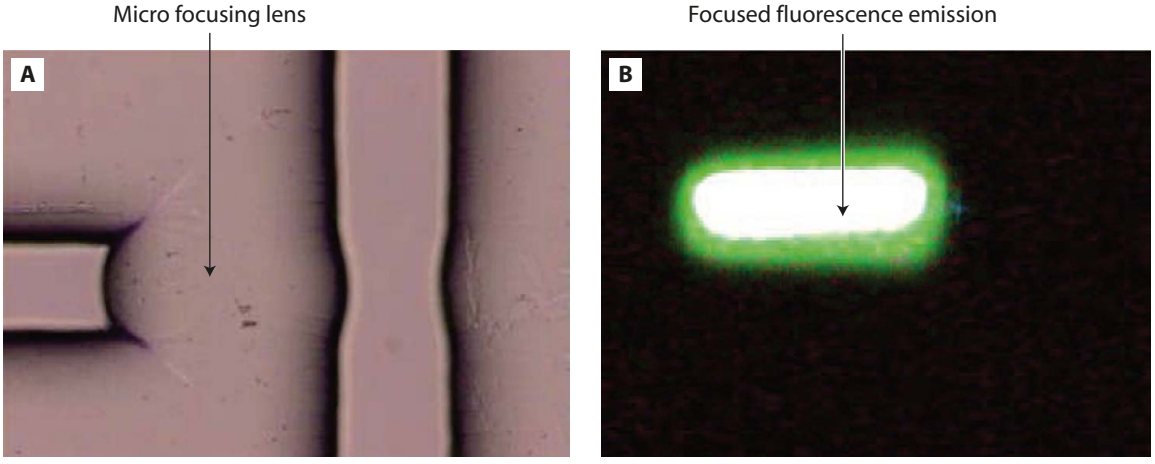

Figure 4 (A) A photograph of the assembled microcapillary electrophoresis chip device integrated with the controllable micro-lens structure. (B) Schematic representation of the experimental setup. Note that detected light sources are transmitted through the two buried optic fibers.

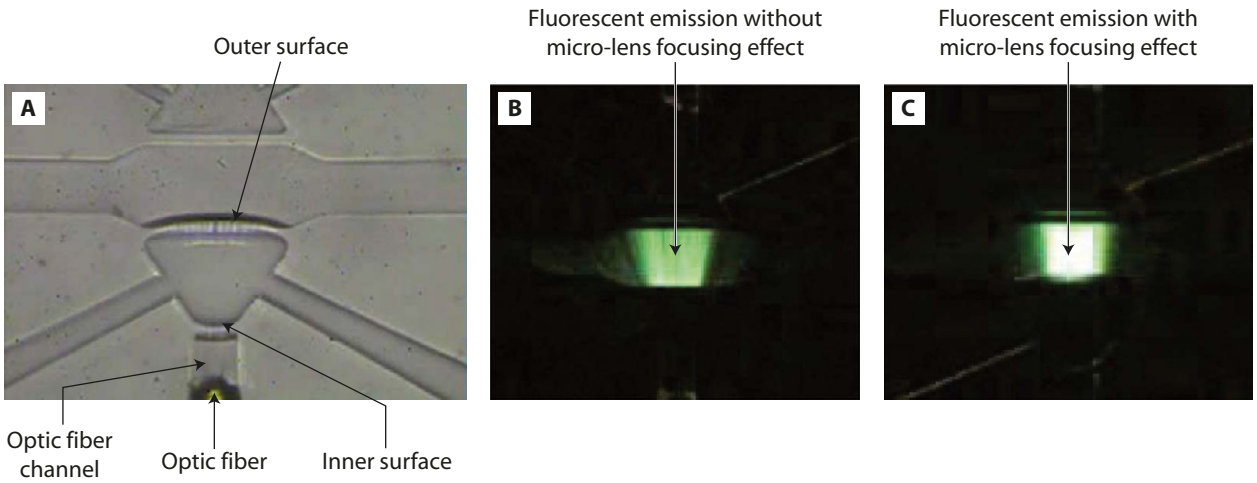

Figure 5 A close-up view of the optical detection area composed of a microcapillary electrophoresis channel, two pneumatic side-chambers, and a pair of optical fiber channels. (A) The micro-lens is activated after the pressurized liquid is injected into the pneumatic-side chambers. Images are shown of the fluorescent emission from 10^{-5}M fluorescein isothiocyanate dye, excited by an argon laser light source transmitted through the optical fiber. (B) The micro-lens is not activated. (C) The micro-lens is activated.

end of the optical fiber and the micro-CE channel. Both the outer and inner surfaces of the micro-lens can be deflected as a double convex lens structure after the index-matching fluid is injected into the pneumatic side-chamber. The curvature of the two surfaces can be actively adjusted by controlling the fluid inlet pressure. Figure 5B shows an image of the fluorescent emission when 10^{-5} M FITC dye was excited by an argon laser light source transmitted through the optical fiber without the deformation of the controllable micro-lens structure. Scattered fluorescent emission can be clearly observed. The results clearly indicated that the fluorescent emission remained unfocused when the deformation of the controllable micro-lens structure was not generated. On the other hand, as shown in Figure 5C, the emission can be focused into a straight beam while the deformations of two surfaces occur, and a higher signal amplitude can be obtained accordingly.

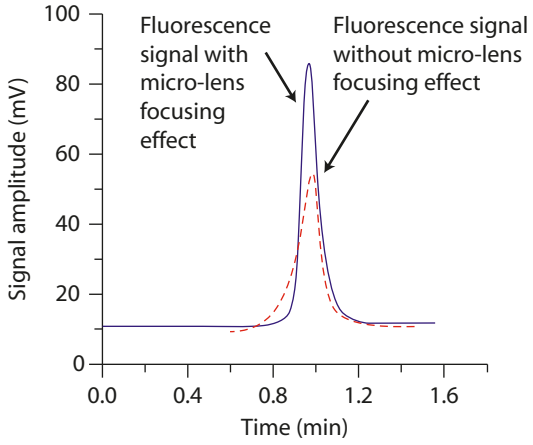

Figure 6 Electropherogram of a fluorescein isothiocyanate sample obtained using two different microcapillary electrophoresis chip designs. The signal amplitude obtained using the fixed focal length micro-lens is significantly higher than when a chip with no focusing capability is used. Specifically, the micro-focusing lenses provide an approximate 1.7-fold improvement.

Discussion

Performance of fixed focal length micro-lens

The fluorescence signals of two FITC (10^{-5}M) sample plugs acquired using two different chip designs—with and without focusing structures—are shown in Figure 6. In both cases, the sample was injected at 0.3 kV over a 0.5-minute loading time and separation was carried out at 0.8 kV for 1.2 minutes. The buffer used in both tests was sodium borate solution (pH 9.2). The signal amplitude of the FITC sample without the focusing effect of the fixed-focal-length micro-lens was obtained in 5.5 mV. Hence, the signal amplitude can be obtained in 8.8 mV by utilizing the integration of the micro-lens structure. Figure 6 shows the signal amplitude, enhanced by an order of approximately 1.7 with the utilization of the proposed micro-focusing lens. Furthermore, with the help of the micro-lens, the detection volume could be as small as $46 \times 120 \times 40\,\mu$m, and could be helpful for reduced band broadening.

In order to discuss the compatibility of biological detection applications, two protein samples were tested to confirm the functionality of the proposed multi-wavelength detection micro-CE chip. We found that the developed microchip device is capable of successfully separating and detecting a protein sample labeled with different dyes. Figure 7 shows an example of a protein sample (β casein) labeled with different dyes (10 ppm FITC and 100 ppm Cy3). As in the previous test, the running buffer was ammonium acetate (pH 6.5). This example shows that the developed microchip can successfully separate and detect the two differently labeled samples. In this test, the Cy3 and FITC dyes are excited by green (543 nm) and blue light (488 nm). The injection of the sample was performed at 0.5 kV for a 1-minute loading time; the separation process was conducted at 1.2 kV for 2 minutes. The results confirm the ability of the micro-CE chip to perform the separation and detection of samples labeled with different dyes by transmitting detection light sources with different wavelengths through the two embedded optic fiber channels.

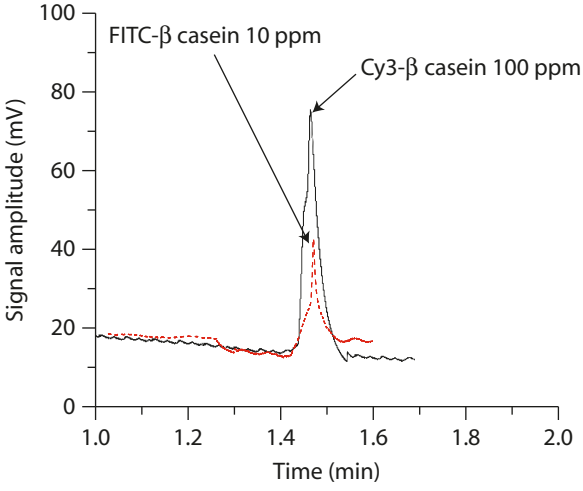

Figure 7 Electropherogram of Cy3 and fluorescein isothiocyanate dye-labeled protein samples (β casein). Note that the Cy3 and fluorescein isothiocyanate dyes are excited by green light (543 nm) and by blue light (488 nm), respectively.

Performance of controllable micro-lens

Figures 8A, 8B show the measured profile of the outer and inner surfaces of the controllable micro-lens structure at different applied pressures. A maximum deformation of the outer surface—as high as 65 μm—can be observed while the index-matching fluid at a pressure of 30 psi is injected into the pneumatic side-chamber. A maximum deformation of 17 μm for the inner surface can also be achieved by using the same pressure as well. After the deformation of the micro-lens structure, the profile of both surfaces can be used as a convex lens to focus the light source.

The activation of the controllable micro-lens can effectively enhance the collected laser power, as shown in Figure 9A. The laser light source is first guided into the micro-CE chip device through the excitation source fiber to the optical detection area. It is then guided out of the chip device through the detection fiber. A commercial optical power meter is utilized for the measurement of the laser power variation. A value of 5 μW is obtained without the micro-lens effect because a major optical

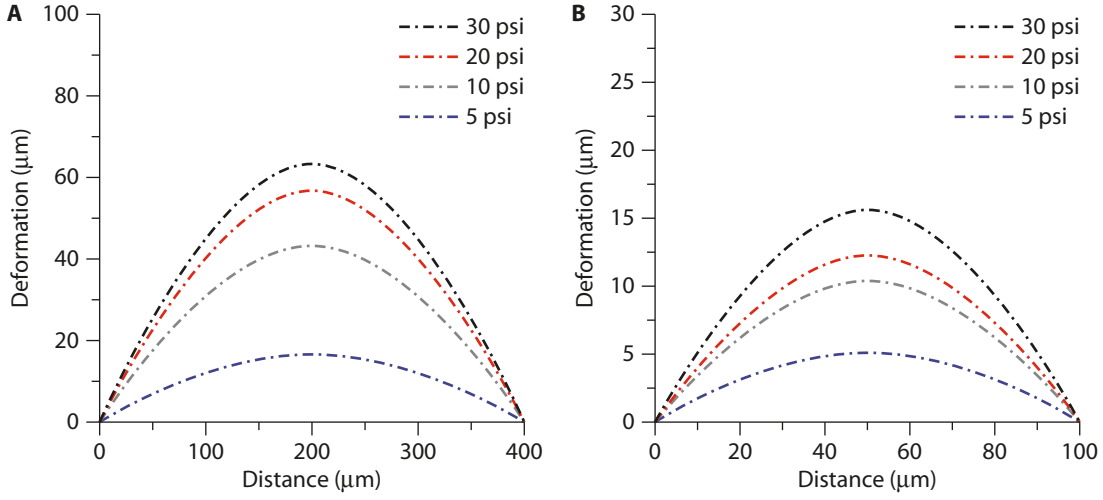

Figure 8 The measured profile of the outer and inner surfaces of the controllable micro-lens structure with different applied pressures for (A) outer surface and (B) inner surface.

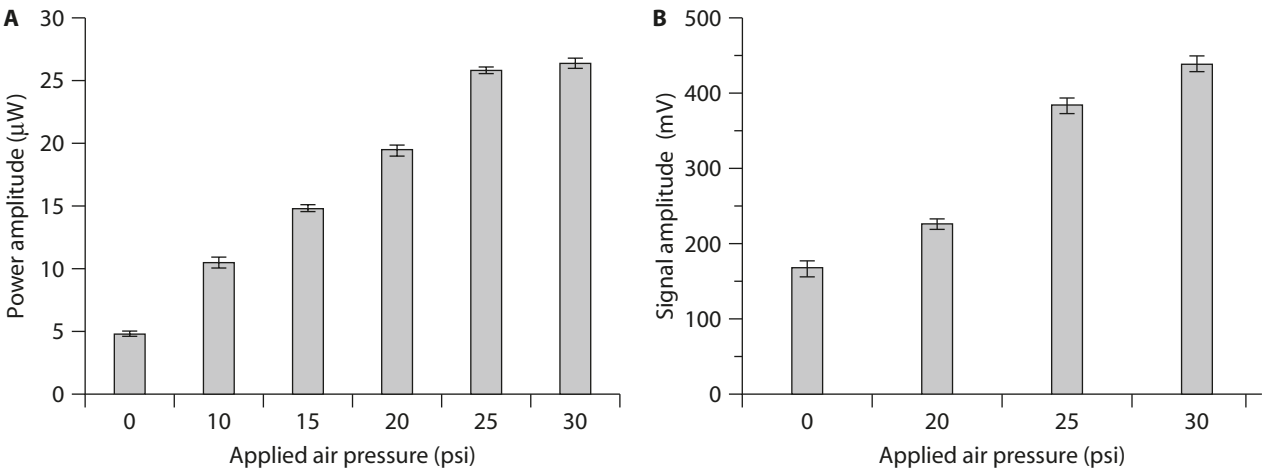

Figure 9 (A) The power amplitude variation of the laser light source with and without the controllable micro-lens effect detected by an avalanche photodiode device. (B) The signal amplitude variation with and without the controllable micro-lens effect detected by an avalanche photodiode device.

loss is generated by the scattering of the excitation laser source in the optical detection area. After the index-matching fluid is injected into the pneumatic-side chamber with a pressure in 30 psi, the optical amplitude can be enhanced to a maximum value of 27 μW.

The signal amplitude with and without the activation of the controllable micro-lens is quantitatively shown in Figure 9B. In order to observe the fluorescent emission, 10^{-5} M FITC dye is filled into the micro-CE channel. The emitted fluorescence can be focused into a straight beam and the optical amplitude can be enhanced effectively by a higher fluid pressure injected into the pneumatic-side chamber when a larger deformation of the micro-lens is obtained. When the surface profile changes with different applied pressures, the signal amplitude of the fluorescent emission can be enhanced accordingly. In this test, the FITC dye is excited by the laser light source guided by the excitation source fiber, and the fluorescence emission is collected by an avalanche photodiode placed on top of the micro-CE chip. The signal amplitude of the FITC fluorescent emission—without the micro-lens—was measured to be approximately 168 mV. The maximum signal amplitude can be as high as 438 mV while the applied index-matching fluid pressure was 30 psi. The experimental results indicate that the fluorescence signal amplitude can be enhanced as high as 2.6-fold when the controllable micro-lens is activated.

Furthermore, in order to demonstrate the capability of the proposed micro-lens structure for biological applications, a separation and online detection of DNA markers (ϕX174 *Hae*III) was performed using the micro-CE chip shown in Figure 10. The diluted DNA sample with a concentration of 2 ng/μL is first loaded into the sampling reservoir, and then electrically driven to the optical detection area to complete the separation process by applying a high voltage between the reservoirs. In order to reduce leakage during the injection process, two methods were employed. First, we precisely pipetted the same volume of CE buffer into each reservoir, to balance the hydraulic pressure between the four reservoirs. Furthermore, during the injection process, two voltages of 200 V and 800 V were applied into the two reservoirs of the separation channel, so that the injected sample flow could be focused and leakage reduced. The data show that DNA markers can be successfully separated into 11 peaks by utilizing the proposed chip device successfully. Moreover, the signal amplitude of the DNA markers can be enhanced 2.5-fold by utilizing the controllable micro-lens structure with an applied pressure of 30 psi. This is in contrast to the signal amplitude, without the enhancing effect.

The experimental results indicated the proposed controllable micro-lens structure is capable of enhancing detection signals and is sensitive to low sample concentrations. Integration of the controllable micro-lens structures with microfluidic chips is a promising method for biological applications.

The present study outlined the development of a micro-CE chip. It incorporates pairs of embedded optic fibers and two different types of micro-focusing-lenses for fluorescence detection purposes—fixed-focal-length and controllable micro-lenses. Experimental results revealed the micro-focusing-lens structure can be successfully deformed as a convex lens to focus the laser light source. Results

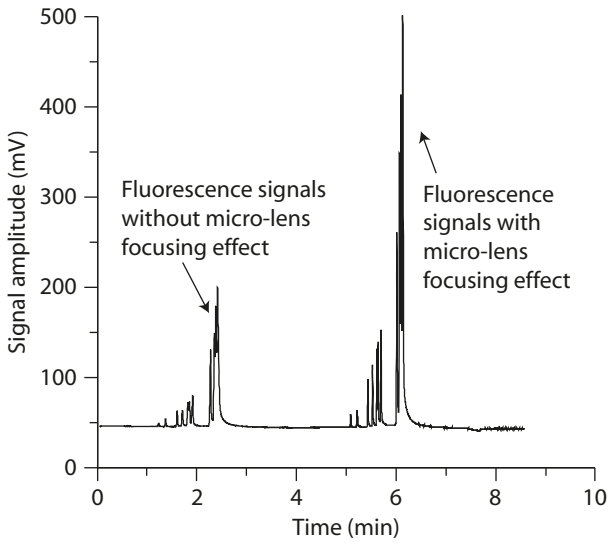

Figure 10 The optical detection result of the DNA marker (A) without and (B) with the controllable micro-lens effect.

also showed that the collected fluorescence signal can be successfully enhanced. The developed device is capable of detecting multiple samples labeled with different types of fluorescent labels in the same channel in a single run. The utilization of fixed-focal-length micro-lenses enhances the signal amplitude of the fluorescence emission by an order of approximately 1.7. On the other hand, we demonstrated a design of a controllable micro-lens device composed of pneumatic side-chambers placed between the micro-CE channel and the optical fiber channel. Experimental results clearly indicated the profile and the focal length of the controllable micro-lens structure can be precisely controlled by using different applied pressures of the index-matching fluid. This means that the power amplitude of the excitation light source can be significantly enhanced, and the amplitude of emitted fluorescence signals from the biosamples labeled by the fluorescent dye can be also enhanced accordingly when the controllable micro-lens structure is activated. Results show the power amplitude of excitation laser light can be enhanced up to 5.4 fold, and the signal amplitude can be enhanced 2.5-fold compared with procedures without a micro-focusing lens. The proposed device has significant potential for use in high-throughput biomedical analysis applications such as hereditary and epidemic disease diagnosis.

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